THE EFFECT OF OXYGEN ON AMOEBA PROTEUS

BY

HAROLD RAYMOND HULPIEU

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H. R. HULPIEU

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[1] Contributions from the Zoölogical Laboratory of the Johns Hopkins University. The problem investigated was suggested by Prof. S. O. Mast and the work was carried out under his directions.

INTRODUCTION

As early as 1774, Bonaventura Corti had observed protoplasmic movements in plants and noticed that this movement ceased after the cells had been immersed for some hours in olive oil. He concluded that the protoplasmic movements of plants are dependent upon air. Since the time of Corti a considerable amount of work has been done on the relation between the amount of oxygen available and the rate of protoplasmic streaming.

Demoor ('95) maintained that streaming in the cells of Tradescantia is stopped by displacing the air with hydrogen and is accelerated by pure oxygen, and that when placed in a vacuum, the effect is the same as that produced by replacement of the air by an inert gas. He obtained the same results in experiments with leucocytes.

Lopriore ('95) came to the conclusion that streaming cannot be stopped in cells of Tradescantia by pure hydrogen and that pure oxygen causes a slight increase in streaming, but he says that this increase is not as marked as had been assumed. Later, he states that pure oxygen does accelerate the streaming, if the streaming is slow, but that pure oxygen has practically no effect on normal streaming.

Kühne ('97–'98) at first maintained that streaming cannot take place without oxygen. He states that by displacement of oxygen by hydrogen, he could stop protoplasmic streaming in Tradescantia stamen hairs, myxomycetes, and amoebae. Later, he asserted that streaming may continue in cells of Tradescantia for a month without oxygen. He concluded that this is due to the presence of an internal store of oxygen.

Clark ('00), working with green plants, fungi, myxomycetes, and a few Infusoria, concluded that streaming may continue for some time in the absence of oxygen.

Davenport ('08) says: "Pure oxygen acts in an opposite fashion from diminished oxygen-tension exaggerating the activity of protoplasm."

Schuster ('13) asserts that there is no adjustment of the streaming, in the cells of Tradescantia, to a lowered oxygen

pressure. He observed an initial increase when the amount of oxygen was decreased, but maintains that this is due to irritation.

From this brief outline of the different views held it will be seen that the state of our knowledge concerning the relation between the amount of available oxygen and protoplasmic movement is very unsatisfactory, and that no definite conclusions can be drawn.

It has often been maintained that the fundamental factors involved in amoeboid activity are essentially the same as those concerned in the contraction of muscle tissue, though found in relation with a much simpler structure. It is well established that an isolated muscle will, under certain conditions, contract for some time in the complete absence of oxygen. This fact indicates that the immediate energy necessary for muscular activity is not derived by a process similar to combustion.

The work of Krogh ('16), Dakin ('22), Meyerhof ('24), Hopkins ('26), Hill ('26), and others has added many other important facts to our knowledge concerning the chemical processes connected with the activity of muscles. Little is known, however, concerning the structural changes occurring during muscular contraction. In Amoeba precisely the opposite obtains. The mechanism of movement has been worked out thoroughly, and little is known concerning the chemical changes involved. For these reasons, it was thought that a study of the effects of oxygen tension in relation to the mechanical processes involved in amoeboid movement would aid in a better understanding of locomotion in Amoeba, and perhaps indirectly, throw light on the mechanism of muscular contraction. With this in mind, the experiments described in the following pages were undertaken.

MATERIAL AND METHODS

Material

The following observations were made on Amoeba proteus (Schaeffer, '16). The stock was obtained originally from a small stream near the university.

Culture methods

Two culture methods were used, one in which the salt content of the water used was unknown (method 1) and one in which the salt content was known (method 2). The experimental results obtained with specimens taken from cultures with known salt content were much better than those obtained with specimens from cultures with unknown salt content.

Method 1. A large number of stock cultures was maintained in the laboratory as follows: Six to eight pieces, about 1 inch in length, of the stem from clean timothy hay were put into about 200 cc. of various proportions of spring, pond, and distilled water. In addition to the hay, one grain of wheat was often added to these cultures. After standing for a few days, the cultures were inoculated with Amoeba and Chilomonas and permitted to develop without further attention. Subcultures were then started from these at intervals of three or four weeks. The hydrogen-ion concentration of these cultures varied from pH 6.5 to 7.0, but it remained fairly constant in any given culture.

The amoebae obtained from these cultures send out pseudopods in all directions, preventing accurate measurements on the effect of oxygen. Moreover, the methods used in ascertaining the effect of oxygen were such that when observations were made with this culture fluid, the hydrogen-ion concentration decreased to such an extent (from pH 6.6 to 7.4 in some cases) that it probably greatly influenced the specimens under observation (Hopkins, '26). These difficulties were overcome by using a buffered culture fluid with a known salt content. Such a buffered culture fluid was used in the second method of culturing amoebae.

Method 2. Hopkins ('26) formulated a modified Ringer solution in which amoebae moved freely and had only one pseudopod, that is, they were monopodal in form. Hopkins' modification of Ringer's solution and several similar solutions were tried, one of which proved very satisfactory.

This solution was compounded from length-of-life data secured from amoebae placed in solutions of pure salts (Mast and Johnson, personal communication). A stock salt solution was prepared by dissolving 4.44 grams $CaCl_2$, 0.356 gram KCl, and 4.48 grams NaCl in redistilled water and diluting to 1000 cc. For use as a culture fluid, 15 cc. of this stock solution was diluted to about 500 cc. with redistilled water, to which was added 22.5 cc. of a phosphate buffer, pH 6.6 (Clark, '25), and the whole made up to 900 cc. This culture fluid will be referred to in the following pages as modified Ringer's solution.

All the amoebae used in the following experiments were cultured for at least five days in this buffered salt solution containing dialyzed hay. The hay was electrically dialyzed, after the method of Hopkins and Johnson ('29), until practically all the inorganic salts were removed.

The amoebae did not multiply rapidly in these cultures, but in cultures five to fourteen days old those present were in excellent condition for experimentation. They were large, fairly clear, and very active. If placed in filtered culture fluid or fresh modified Ringer's solution, streaming was practically monopodal.

Chemicals

The water used in the modified Ringer's solution was first distilled from a block-tin still; and then carefully redistilled from a tandem still, made entirely of pyrex glass, and consisting of three vaporization chambers and a trap, all in series. The first chamber contained a weak solution of sulphuric acid and potassium permanganate in water from the block-tin still; the second, an excess of barium hydroxide, and the third, water that had passed through the first two

chambers (Mast, '28). The redistilled water thus prepared had, owing to the carbon dioxide absorbed from the air, a hydrogen-ion concentration of about pH 6.2.

The salts used in the solutions were the purest obtainable and were carefully recrystallized either in the laboratory or by Hynson, Wescott & Dunning, Baltimore.

Hydrogen-ion determination

The method of ascertaining the hydrogen-ion concentration was similar to the La Mott method for small amounts of fluid (Brown, '24), which has the advantage that less than 1 cc. of fluid is required to make an individual test, and besides being very rapid, is accurate to one-tenth pH.

Apparatus

The amoebae were subjected to various oxygen tensions by means of a modified Englemann gas chamber, designated an observation chamber (fig. 1). It was constructed entirely of

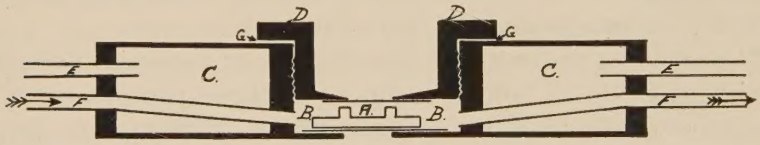

Fig. 1 Diagrammatic vertical section of the observation chamber *A*, cell of pyrex glass for holding the amoebae; *B.B.*, gas chamber; *C.C.*, water compartment for temperature control; *D.D.*, depression plug for closing gas chamber; *E.E.*, water intake and outlet; *F.F.*, gas intake and outlet; *G.G.*, position of magnet.

brass, with the exception of flexible copper tubing (*f*) used to conduct the different gas mixtures to and from the gas compartment (*b*). The water compartment (*c*) was designed for accurate temperature control, but was not used in the present work. The depression plug (*d*), which closed the gas compartment, permitted the amoebae to be brought within the focal distance of the microscope. This plug was threaded and its shoulder pressed on a rubber gasket which sealed the

chamber. The glass top and bottom were sealed with De Kotinsky cement. It was found that the cement was slightly toxic; consequently, all exposed cement was carefully covered with a hard paraffin. When this was done, the poisonous effects were eliminated.

EXPERIMENTAL METHODS AND TEST OF APPARATUS AND MATERIAL

Experimental methods[2]

A considerable number of amoebae were selected from cultures in modified Ringer's solution, washed fairly free from organisms, and put into a fluid consisting of about three-fourths fresh modified Ringer's solution and one-fourth filtered fluid from the culture. Amoebae treated in this fashion assumed a nearly monopodal form and moved at a fairly constant rate for as long as two or three days. Six of these washed animals, with about four drops of solution, were then put into each of two small glass containers.[3] One of these was put into the observation chamber, and the other was put into a damp chamber with an excess of air as a control. A stream of moist air was passed slowly through the observation chamber. The animals in the chamber were then compared with the control animals in respect to activity, structure, and appearance.

The rate of locomotion was taken as a measure of the activity. This was ascertained by projecting an image of an amoeba on a sheet of paper by means of a camera lucida and marking the position of the posterior end at one-minute intervals.[4] In each test the three most active individuals of the six put into the observation chamber were selected; then the distance traveled in five minutes was ascertained for each of

[2] All experiments described in this and the following sections were conducted at room temperature, which varied from 21° to 24°C.

[3] These containers consisted of a ring (about 1 cm. in diameter and 0.2 cm. high) cut from pyrex-glass tubing and fused to a flat plate of pyrex glass (fig. 1, *A*).

[4] The image as projected upon the paper was magnified 146 diameters, making the rate obtained 146 times the actual rate.

them and the average rate per minute calculated. The control specimens were treated in the same way.

A brief description of the structure of Amoeba under normal conditions is here presented as an aid in understanding the methods used in ascertaining the changes occurring in amoebae under the different conditions.

> Amoeba proteus contains a central elongated fluid portion (plasmasol), a rigid layer surrounding this (plasmagel), a thin elastic surface layer (plasmalemma), and a hyaline layer between the plasmagel and the plasmalemma which is fluid at the tip of active pseudopods and in certain other regions.
>
> During locomotion . . . . the plasmasol continuously gelates at the tip of the extending pseudopods forming plasmagel, and the plasmagel continuously solates at the posterior end forming plasmasol (Mast, '26).

The relative amount of the plasmagel and the plasmasol varies when the animal is subjected to different conditions; under one set of conditions the stream (plasmasol) is very wide and the plasmagel consequently very thin, while under other conditions this relation is reversed.

The relative amount of plasmagel and plasmasol present for any given set of conditions was approximately determined by measuring the width of the animal and that of its stream in optical cross-section. These measurements were made with the aid of a camera lucida in much the same manner as the rate of locomotion described above. Then by subtracting the width of the plasmasol from the entire width of the animal, the width of the plasmagel was calculated. The width of the plasmagel was then divided by the width of the plasmasol. The number thus obtained was called the gel/sol ratio. This ratio was ascertained at the same time as the rate of locomotion and, whenever possible, for both the posterior and anterior ends of the same animal. The six results which were obtained in the above manner were then averaged, giving an average ratio which is a good index of the relative amount of gelation and of the condition of streaming.

Results

The results obtained from one test made in the manner described above are presented in figure 2. These curves show that locomotion, streaming, and changes in structure of the specimens in the observation chamber were practically the same as those in the damp chamber. This demonstrates that the observation chamber contains nothing injurious to Amoeba.

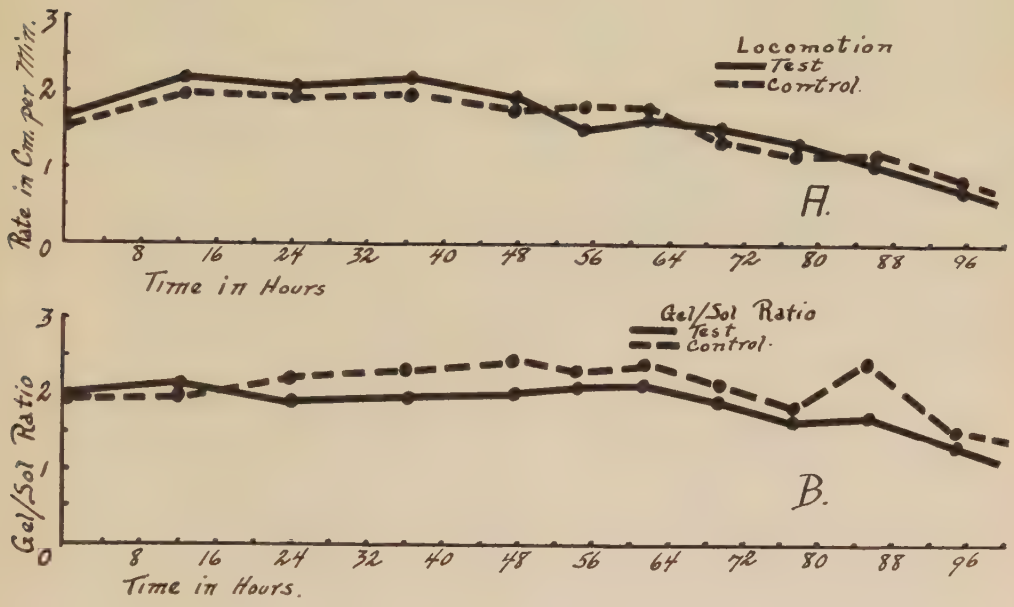

Fig. 2 Effect on Amoeba of long confinement in the observation chamber.

By referring to figure 2, it will be seen that the rate of locomotion of both the control and the experimental animals remained practically constant for about sixty hours, then gradually decreased, reaching zero at the end of about one hundred hours, when all the animals were either dead or completely rounded. After about eighty hours, it was not always possible to find three animals giving good locomotion; therefore some of the points upon which the curve was based were established from data from one or two animals.

By comparing figure 2, A, with figure 2, B, it will be seen that the gel/sol ratio remained practically constant in both the experimental and the control animals over a period of sixty hours and then decreased, indicating that a rapid rate of locomotion is correlated with relatively little plasmasol and slow movement with relatively much plasmasol.

In a second test (data not given) very similar results were obtained to those in the first test, with the exception that the animals confined in the observation chamber ceased locomotion and became very fluid about ten hours earlier than those in the control. Complete cessation of locomotion occurred at about seventy hours in the observation chamber and at about eighty hours in the control. The gel/sol ratio decreased at practically the same time and at almost the same rate as did the rate of locomotion.

After the animals in both the control and the chamber had become rounded, they were removed to a moist chamber. Two and one-half days later, quite unexpectedly, it was discovered that two animals in each had recovered. All the remaining amoebae had disappeared. The bottom of the dish was covered with a bacterial film, and several specimens of Chilomonas were observed, whereas before there had been, at most, only one or two present. This would seem to indicate that either the modified Ringer's solution lacked some essential protein which was supplied by the animals that disintegrated, or that these animals furnished food for the bacteria which then either changed the solution chemically or indirectly furnished food for the amoebae that were able to survive.

In both of these two tests the amoebae in the control were very similar in external appearance to those in the observation chamber. A brief description of the changes in structure and form that occurred in practically all the animals in these two tests follows.

Amoebae in fresh modified Ringer's solution assume a more or less monopodal condition (fig. 3, A). Animals in this condition have a fairly constant rate of locomotion for a con-

Fig. 3 Camera-lucida drawings illustrating typical changes in structure and form occurring in amoebae in modified Ringer's solution, in a small glass vessel. The time required for the appearance of the different forms varied greatly, thus the time given below is only an approximation. The drawings refer to changes observed in form, and the shading is not an attempt to picture granules, but only their distribution. A. Appearance of amoebae after a few hours in modified Ringer's solution. B. Appearance of amoebae after about sixty hours; plasmagel thinner, locomotion good. C. Appearance of amoebae after about seventy hours; plasmagel very thin, streaming much slower, locomotion slower, sudden changes in direction of movement by breaking out of large pseudopods. D, E, and F. Appearance of amoebae after about eighty hours; practically no streaming, very sluggish irregular movements of granules, animals rounding up, no plasmagel could be discovered. G, H, and I. Disintegration or cytolysis of one of the amoebae; five-minute intervals between consecutive drawings.

siderable time. If, however, they are subjected to this solution for a sufficient length of time, they come to resemble the one illustrated in figure 3, B. The rate of locomotion appears to be correlated with the thickness of the plasmagel. In this and the following tests the plasmagel almost always became thinner at the same time or before any noticeable decrease in the rate of locomotion. The amount of plasmagel becomes less and less, until the animal no longer maintains locomotion in a definite direction. Direction of flow then often changes suddenly, owing to large areas of the plasmagel giving way and resulting in the formation of extremely large lateral pseudopods (fig. 3, C). After this, the rate of streaming decreases until not even slight movements of the granules can be observed, then the anterior end of the animals very slowly enlarges and gradually the whole body flows into this enlargement until a spherical shape is assumed (fig. 3, D, E, and F). Often, after the animals have become completely rounded, the plasmalemma breaks and the whole animal goes into solution (fig. 3, G, H, I). Frequently, the amoebae are overtaken by death before they are completely rounded. When this occurs, they become light yellow in color and appear to be coagulated.

This series of reactions or changes in form is indicative of unfavorable conditions, and if not checked by a change in the environment, it always results in death. This fact is brought out more clearly in the following series of experiments in which changes were made in the amount of available oxygen. Because the amoebae in these tests and in all the following experiments passed through the same series of forms a few hours before death, the condition of the animals was called pathological, and hence the terms pathological condition and pathological forms will be used in the following pages in referring to this series of changes.

The rate of division of the control and the experimental animals were practically the same in these tests. Six of the control and five of the experimental animals divided during the course of the two tests described above.

The following conclusions were drawn from these tests: 1) Animals confined in the observation chamber behaved in the same manner as those kept in a moist chamber, showing that there were no injurious substances in the observation chamber. 2) The rate of locomotion, the gel/sol ratio, and the general appearance were the same for both the control and experimental animals, and they remained fairly constant for about sixty hours. 3) The rate of locomotion varied directly with the gel/sol ratio. 4) The rate of division was the same in amoebae under the two conditions.

EFFECT OF INCREASING THE AMOUNT OF AVAILABLE OXYGEN

Methods

The effect of increasing the amount of available oxygen was ascertained as follows: Amoebae were washed and mounted in the observation chamber as described above. The air in the observation chamber was then replaced by slowly passing through it oxygen from a tank. Observations were then made on the rate of locomotion, the structure, and the external appearance, in the same manner as in the previous tests. The pressure of the oxygen was regulated by means of a Hoke Phoenix pressure gauge, and the rate at which the gas passed through the observation chamber was regulated either by means of a Schrader valve or a water seal.

Three series of experiments were made; in the first the oxygen was under a pressure of 1 atmosphere (0.00 pounds)[5]; in the second, 2.709 atmospheres (25 pounds), and in the third, 4.418 atmospheres (50 pounds).

Oxygen, 1 atmosphere

Three separate tests were made in this series, but because of their similarity the curves obtained for only one are given (fig. 4).

[5] The pressure given here, in pounds, is not in respect to an absolute vacuum, but is the number registered by the Hoke pressure gauge. Zero on this gauge is equal to a pressure of 1 atmosphere.

By referring to figure 4, A, it will be seen that the rate of locomotion in oxygen at atmospheric pressure was for fifty hours essentially the same as that in air, but that from then on it decreased rapidly in oxygen and only slightly, if at all, in air. It will also be seen that after the oxygen in the observation chamber was replaced by air, the rate of locomotion increased until at the end of 120 hours it was a little higher than that in the control. However, only two of the animals treated with oxygen survived for a period of one hundred hours and only one had completely recovered by the end of 120 hours, while two of the controls were streaming normally at the end of this period. All the remaining animals had disappeared. It is, consequently, evidence that the difference in the results obtained, under the two conditions tried in this test, is slight but definite.

By comparing the two curves in figure 4, it will be seen that the gel/sol ratio of the specimens in oxygen decreased as the rate of locomotion decreased and increased as the rate of locomotion increased, but that the decrease and the increase in the former preceded the decrease and the increase in the latter.

These facts seem to indicate that oxygen at a pressure of 1 atmosphere has, for a period of fifty hours, little if any effect different from air, but for longer periods it causes a decrease in activity. They also indicate that rapid locomotion is correlated with relatively a large amount of plasmagel and slow locomotion with relatively a small amount of plasmagel.

A second test continued for only a little over fifty hours showed that during this time the rate of locomotion and the gel/sol ratio were essentially the same in oxygen as they were in air. The test was discontinued, owing to the sudden unexplainable death of all the specimens in both the control and the observation chamber. The results obtained in this test are, over the period it extended, in full harmony with those obtained in the first test.

In a third test the rate of locomotion in oxygen was, for a period of thirty-five hours, about the same as in air, but it then began to decrease rapidly, reaching zero at the end of

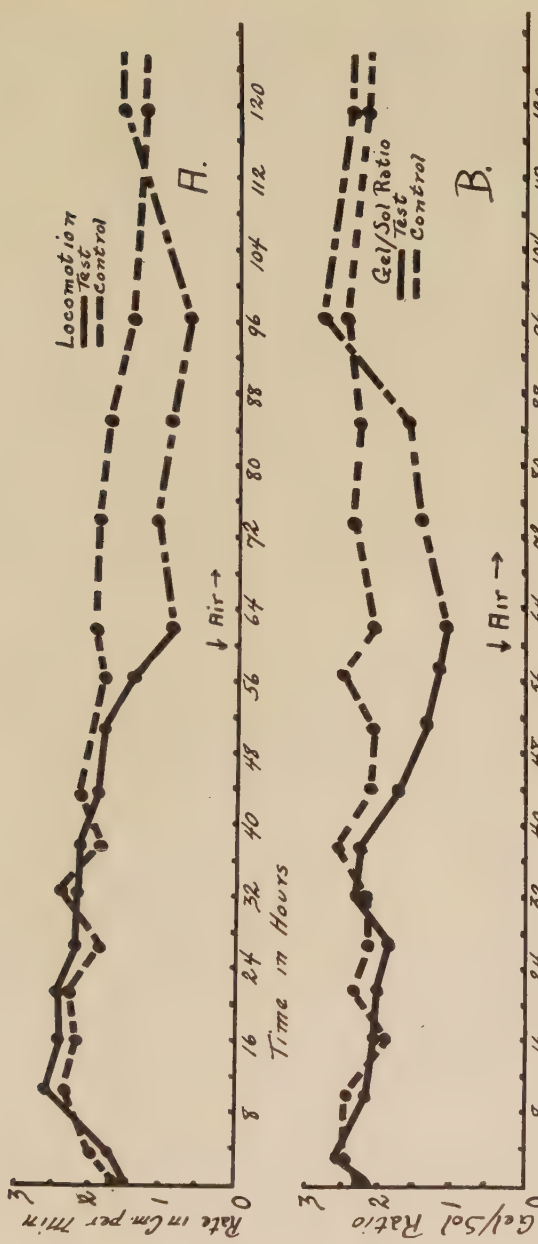

Fig. 4 Effect of oxygen at 1 atmosphere on Amoeba. The pressure of oxygen is given in respect to an absolute vacuum, therefore 1 atmosphere means that the air was replaced in the chamber by pure oxygen. This holds for the other experiments with increased oxygen tension.

thirty-eight hours. These animals did not recover when restored to air. When observed at about forty hours, streaming had decreased to such an extent that the gel/sol ratio could not be ascertained. These results indicate that oxygen, at atmospheric pressure, after a period of about thirty-five hours greatly decreases the rate of locomotion in amoebae.

In all three tests a series of changes occurred in all the animals, except the controls in the third test. These changes were similar to those described in the preceding section and there designated pathological changes. In this respect there was no marked perceptual difference in the animals in oxygen and those in air, except in the second test. However, only one division occurred in the oxygen-treated animals in all three tests, as against eight divisions in the control animals.

The results of this series of experiments, taken as a whole, clearly indicate that increasing the oxygen tension from $\frac{1}{5}$ to 1 atmosphere has a slight detrimental effect on Amoeba.

Oxygen, 2.709 atmospheres

Four separate tests were made in this series; the results from a typical test are presented in figure 5.

It can be seen by an examination of figure 5 that the rate of locomotion in the oxygen remained the same as that in the air for about sixteen hours; that it then began to decrease, and continued until the oxygen was replaced with air, after which it increased until the close of the test, when it was only slightly lower than that in the specimens in air. When the gel/sol ratio is compared with the rate of locomotion, it is clearly seen that a significant decrease in the gel/sol ratio of the amoebae in oxygen occurred about four hours earlier than the decrease observed in the rate of locomotion, and that after the oxygen was replaced by air, this ratio increased until it was a little lower than that in the control. Typical pathological forms were observed, beginning at about the same time that the increase in the gel/sol ratio became significant.

Similar results were obtained in a second test; the only difference being that the decrease in the gel/sol ratio began at about the same time as the decrease in the rate of locomotion, not earlier as in the first test.

In tests 3 and 4 the gel/sol ratio was not ascertained. The rate of locomotion in these tests decreased after about the same length of exposure and at about the same rate as in the two preceding tests. After the oxygen was replaced by air,

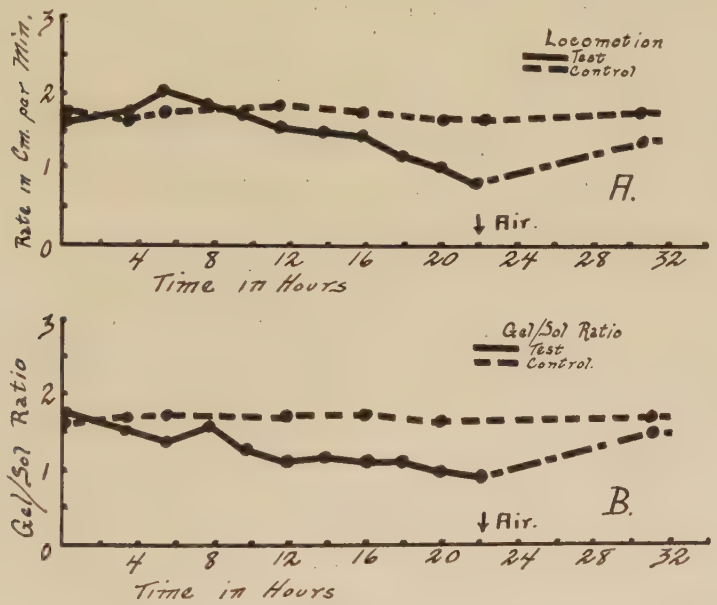

Fig. 5 Effect on Amoeba of oxygen at 2.709 atmospheres.

three specimens in test 4 regained their former rate of locomotion, but those in test 3 did not. These specimens rounded up, became inactive, and died without recovering.

During these four tests, division did not occur in the animals in oxygen, nor did it occur in these animals after restoration to air, but eight divisions occurred in the control animals.

It was concluded that the same effects are produced by oxygen at a pressure of 2.709 atmospheres as that produced in oxygen at a pressure of 1 atmosphere, but in a much shorter time.

Oxygen, 4.418 atmospheres

Seven separate tests were made in this series. In the first two tests, the gel/sol ratio and the rate of locomotion were ascertained, while in the remaining five tests this ratio was not ascertained. Figure 6 illustrates the results obtained.

By referring to figure 6, A, it can be seen that, in the first test the rate of locomotion began to decrease rapidly after the animals were exposed to the oxygen, and that this continued until the oxygen was replaced by air. After this, the rate of locomotion was not ascertained. But about six hours after the oxygen was removed, streaming was 'very slow' in the animals in the oxygen and 'good' in those in the damp chamber. At the close of the test, four of the specimens, which had been in oxygen, had completely recovered and the rest had disappeared.

It was thought that the decrease in rate of locomotion observed in oxygen might perhaps have been due to some alteration in the modified Ringer's solution produced by the oxygen, and not to the action of the oxygen directly on the amoebae. Consequently, three specimens were taken from the control and put into the fluid in the observation chamber after it had been subjected to oxygen at a pressure of 4.418 atmospheres for thirty-six hours and the experimental animals had been removed from it. These three specimens were then compared with those which remained in the control vessel. No difference was observed, indicating clearly that the decrease in rate of locomotion, observed in the oxygen, was due directly to the action of the oxygen on the amoebae.

By comparing A and B in figure 6, it will be seen that in this test, as in the tests with oxygen at a pressure of 1 atmosphere, a decrease in the gel/sol ratio occurred a short time preceding the decrease in the rate of locomotion, and that this ratio was but slightly, if at all, affected by putting the control animals into oxygen-treated fluid. The amoebae in oxygen in this test passed through the pathological conditions described above for the tests with air.

In the second test the rate of locomotion of the specimens in oxygen began to decrease almost immediately after being subjected to oxygen, and it reached zero at the end of about twenty-three hours. The specimens used in this test did not recover when restored to air. The gel/sol ratio remained practically the same as that in air for about eleven hours, then decreased very rapidly. Thus the gel/sol ratio did not begin to decrease until several hours after locomotion had

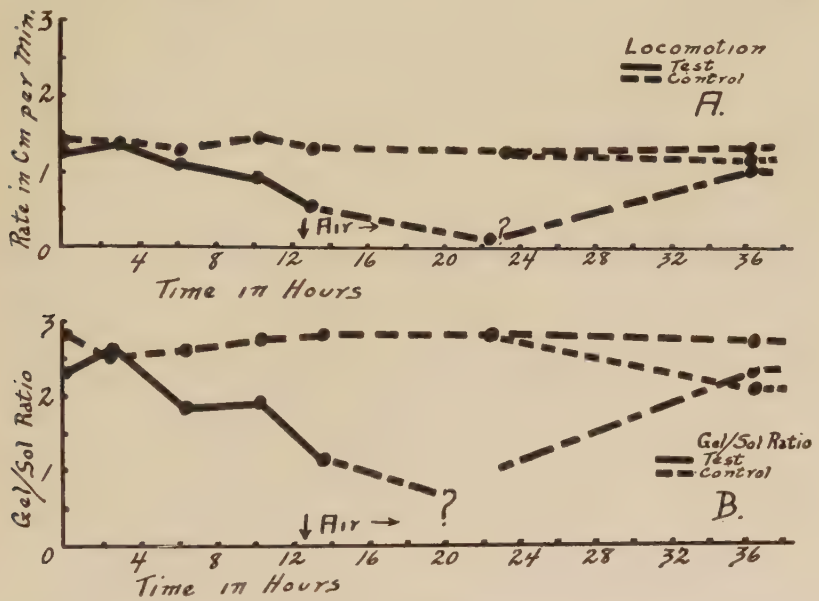

Fig. 6 Effect on Amoeba of oxygen at 4.418 atmospheres.

been retarded. This is precisely opposite to that observed in almost every other test. In other respects the results obtained in the two tests were the same.

The next two tests were made with the same animals, but on different days. During the first of these tests the rate of locomotion in oxygen decreased rapidly until the oxygen was replaced by air, after which it increased rapidly until it was slightly higher than that of the controls. In the second test exposure to oxygen was continued until the effects were more severe. In this test the rate of locomotion decreased rapidly in oxygen, but recovery was very slow.

Changes in the rate of locomotion similar to those described above were obtained in the three remaining tests. In one of these tests the amoebae were kept, with air, in the observation chamber for a period of twelve hours before the oxygen was introduced. In the last test the control animals were put into the fluid in which the experimental animals had been during the test. This exchange was made immediately after the removal of the oxygen from the observation chamber. The effect of the oxygen had been pronounced, the animals that had been in the observation chamber having died. This solution, however, had no injurious effect on the controls, showing again that retardation in the rate of locomotion observed in oxygen was due to a direct action of the oxygen on the amoebae.

In this entire series of tests no divisions occurred in animals in oxygen, while nine divisions occurred in the specimens kept in air.

The following was concluded from these tests: 1) The effect of 4.418 atmospheres of oxygen on the rate of locomotion, the gel/sol ratio, and the appearance of amoebae is like that produced by 1 atmosphere of the same gas, except that a much shorter time is required. 2) Recovery does not take place after the animals are restored to air, if the effects of the oxygen have been too severe. 3) The process of division is stopped by oxygen at this pressure. 4) The effect of oxygen on amoebae is due to a direct action of the oxygen on the organism, and not to changes in the culture fluid.

EFFECT OF DECREASING THE AMOUNT OF AVAILABLE OXYGEN

Methods

The amount of available oxygen was decreased with nitrogen or hydrogen, or a mixture of nitrogen and oxygen. Four series of experiments were performed, in which the air in the observation chamber was replaced with nitrogen containing about 0.5 per cent oxygen (tank nitrogen), i.e., oxygen at a pressure of approximately 0.005 atmosphere, one in which it was replaced by nitrogen which contained only a trace of

oxygen (tank nitrogen passed through pyrogallol), one in which it was replaced by nitrogen which contained practically no oxygen (tank nitrogen passed over heated copper), and one like the preceding, except that hydrogen was used in place of nitrogen. It was necessary to use different methods of handling these gases in each of the four series of experiments; consequently, these methods will be described in connection with each series of experiments.

The nitrogen used was "Airco" nitrogen purchased from the Air Reduction Sales Co., of New York. This company has very kindly supplied the following data.

> Airco nitrogen is guaranteed to be 99.5 per cent pure while the actual quality supplied is in the neighborhood of 99.7 per cent to 99.8 per cent. The impurity is oxygen. Qualitative tests show that there are spectroscopic traces of neon, helium and hydrogen present.
>
> Airco nitrogen is supplied either water-pumped or oil-pumped. Both are free from acid and dust and the former, while free from hydro-carbon vapor will contain a trace of moisture, whereas the latter is dry but contains a trace of hydro-carbons.

This analysis of the tank nitrogen shows that the only impurity present known to have any effect on living organisms is oxygen. In the experiments, which did not require absence of oxygen, the tank nitrogen was merely passed through redistilled water. A slight film of oil on the surface of water is known to affect greatly the time required to obtain equilibrium between gases above the water and those in solution. For this reason, the traces of hydrocarbons were removed by washing the nitrogen with concentrated sulphuric acid before passing it through the wash tube containing redistilled water.

Oxygen at 0.005 atmosphere (tank nitrogen)

In the first series of experiments the air was replaced with tank nitrogen by means of an apparatus very similar to that described in the experiments with oxygen at a pressure of 1 atmosphere.

Two separate tests were performed with this concentration of oxygen, the results of one of which are shown in figure 7. Figure 7, A, shows that, for the first fifty to fifty-five hours, the rate of locomotion was the same in both the experimental and the control animals, but that it then decreased in the animals in nitrogen, and that it was increased only slightly, if at all, when the nitrogen was replaced by air. At the end of one hundred hours, all the animals in both the control and the observation chamber were dead.

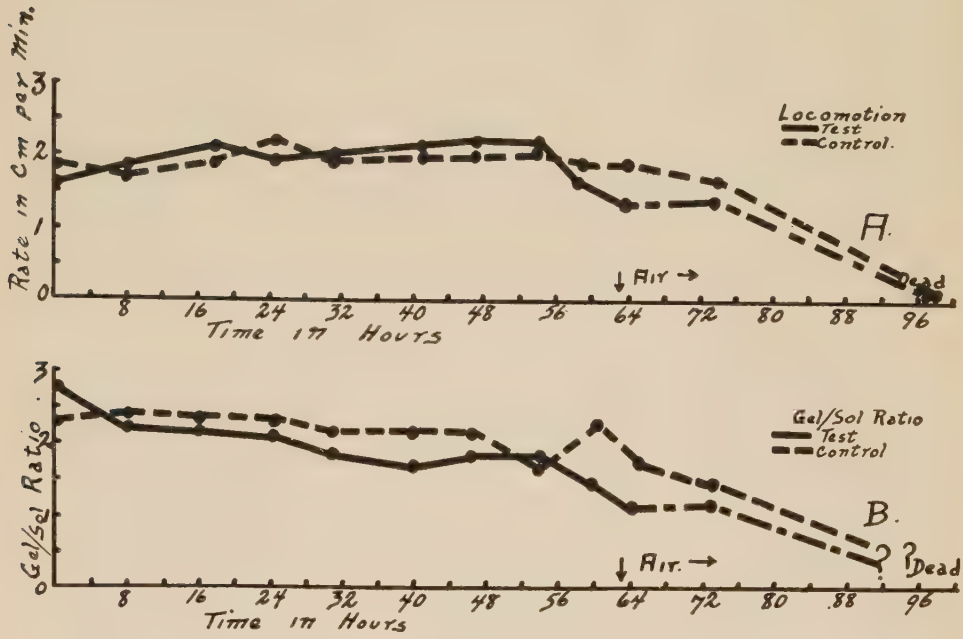

Fig. 7 Effect on Amoeba of decreasing the oxygen partial pressure to 0.005 atmosphere.

When the gel/sol ratio of these animals (fig. 7, B) is compared with their rate of locomotion, it is clearly seen that this ratio, after an initial decrease, followed the rate of locomotion very closely, i.e., that a slight increase in locomotion after forty-five to fifty hours was accompanied with a slight increase in the gel/sol ratio. These results point with remarkable clearness to a correlation between activity and the thickness of the plasmagel.

The changes occurring in these animals in respect to structure and appearance were almost identical with those described above and designated pathological. These changes occurred in both the control and the experimental animals.

The same results were obtained in the second test, with the exception that four of the controls were alive at the end of one hundred hours, while all the experimental animals were dead. However, two of these four animals were in the later stages of the typical pathological condition, showing that the difference between the animals in nitrogen and in air was not very great.

The rate of division was not changed by this reduction in the oxygen tension; three of the experimental and four of the control animals divided during the course of these two tests.

The results of this series of experiments, taken as a whole, indicate that decreasing the oxygen tension from 0.2 to 0.005 atmosphere has only a slight detrimental effect on amoebae.

Oxygen at very low pressure

In this series of tests the air was expelled from the observation chamber by means of purified nitrogen. The nitrogen used was led through sulphuric acid, then passed through three wash tubes containing an alkaline solution of pyrogallic acid, and, finally, washed with redistilled water. The oxygen was in all probability not completely removed from the nitrogen by this method.

Olsen ('22) makes the following statement in regard to absorption of oxygen by pyrogallol:

> The absorption is not complete unless a large excess of the reagent is present. When the solution has absorbed a few percent of the amount of oxygen which it will take up, it leaves, during subsequent use, a larger and larger residue of unabsorbed oxygen in the gas which is in contact with it.

Hutchins ('25), who employed this method of removing oxygen from nitrogen, exercised the utmost precaution. He

removed the final washing solutions as soon as the least trace of color appeared. The solutions of pyrogallol used in the present work turned a deep brownish-black after a few hours, but were not removed. Thus it is quite evident that all the oxygen was not removed. The exact amount that remained was not ascertained, but it was considerably less than was present in the tank nitrogen.

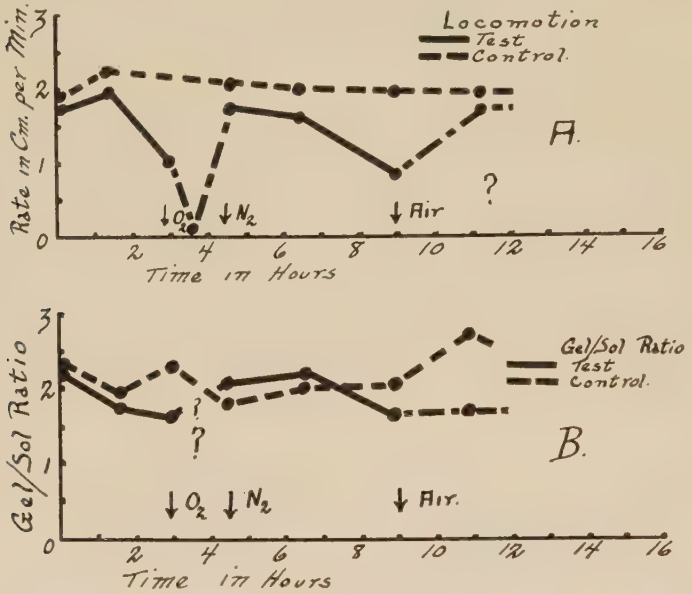

Fig. 8 Effect on Amoeba of decreasing the partial pressure of oxygen below 0.005 atmosphere.

Three separate tests were made in this series of experiments. In the first test the rate of locomotion, in the animals exposed to the nitrogen used, decreased very rapidly, until it had fallen below 1, as shown in figure 8, A. Oxygen was then admitted to the observation chamber. This was followed by a very interesting series of phenomena (fig. 9).[6]

[6] This series of phenomena did not occur in all the amoebae observed, but it did occur in a sufficiently large number of each group examined to be considered as a characteristic reaction. It was also observed in the following experiments with oxygen-free nitrogen and hydrogen.

In one to five minutes after the oxygen entered the observation chamber, an increase in the movements of the granules occurred, resulting in a very slow streaming. The animals then slowly settled to the bottom and became attached, after which they flattened until they became very thin, transparent sheets (fig. 9, E, F, and G). After remaining in this condition for ten to twenty minutes, they began to stream in all directions, until one pseudopod finally gained the lead; then the whole animal moved in a practically monopodal form. This reaction will be referred to in the following pages as the reaction to oxygen after oxygen-want. It is represented in figure 8, A, by a broken line, which indicates that the rate of locomotion fell to zero after the admission of oxygen.

After the animals had become normal in behavior and appearance, the oxygen was again driven out with nitrogen, which resulted in a rapid decrease in the rate of locomotion. The response to oxygen after oxygen-want was not observed in the last part of the test, although it may have occurred. No difference was detected in the length of time required for recovery in air and in oxygen.[7]

The gel/sol ratio (fig. 8, B) also decreased and increased at about the same rate as the rate of locomotion, but the change in this ratio was relatively not as great as had been observed in previous experiments, and it, furthermore, was exceedingly difficult to obtain, because of rapid changes and irregularities in streaming. The gel/sol ratio of the controls also showed great variations, so that no definite conclusions can be drawn from this part of this experiment.

In the second test the rate of locomotion decreased rapidly and reached zero at the end of six hours, after which, when the animals were restored to air, it increased even more rapidly. The gel/sol ratio exhibited characteristic changes, increasing and decreasing with the rate of locomotion.

The results obtained in the third test were essentially the same as those obtained in the first two tests. The response to

[7] No difference could be observed in the recovery of experimental animals in air, oxygen, carbon-dioxide-free air, or carbon-dioxide-free oxygen.

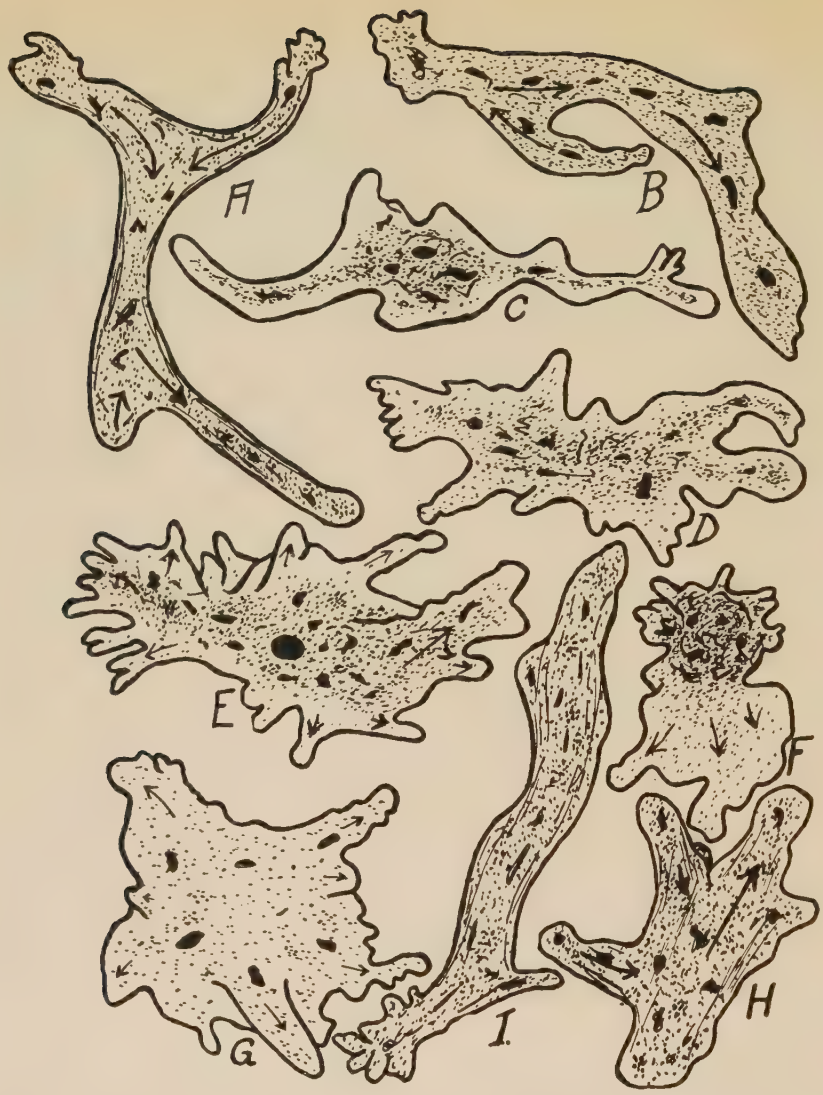

Fig. 9 A series of camera-lucida drawings showing progressive changes in form, external appearance, and the distribution of granules occurring in amoebae when they are exposed to oxygen or air after subjection to an insufficient oxygen supply. A. Floating free, without locomotion, irregular movement of granules possibly the result of insufficient oxygen. B. First reaction to oxygen, increased movements of granules resulting in more rapid change in form, no attachment. C. Attached, somewhat flattened, protoplasm clear at edges, very little movement of granules. D and E. Very firmly attached, flattened to a thin layer, clear at edges, no movement of granules. F. Firmly attached, very slow movement of granules, granules collecting in a definite location. G and H. Streaming beginning, but as yet in various directions, attached. I. Complete recovery.

oxygen after oxygen-want occurred upon the admission of air. It would appear from our observations that this response occurs equally as well with air as with oxygen, but that it will not appear if the lack of oxygen has not been continued sufficiently long, or if it has been continued too long.

In these three tests the control specimens remained in practically a monopodal condition throughout, but typical pathological forms were observed in the experimental animals, although these were only early stages.

The rate of division was reduced to zero by the decrease in the amount of available oxygen; not one of the experimental animals divided, while three divisions occurred in the control chamber.

The following conclusions were drawn from the results obtained in these three tests: 1) Oxygen pressure considerably lower than 0.005 atmosphere produces a very rapid and decided decrease in the rate of locomotion in Amoeba, after which recovery in air or oxygen is equally rapid. 2) The gel/sol ratio and the external appearance of the animals are only slightly affected. 3) Amoebae give a very characteristic response to oxygen after a period of oxygen-want. 4) The rate of division in reduced oxygen pressure decreases greatly.

Oxygen-free nitrogen

The oxygen was removed from the nitrogen used in this series of experiments by passing the tank nitrogen through red-hot copper gauze which had previously been reduced by hydrogen. The apparatus was made of pyrex glass and copper tubing. All glass joints were sealed by fusion, and all metal joints were carefully soldered. There was only one rubber connection in the line between the furnace and the observation chamber. This joint connected the copper tube from the observation chamber with the glass tubing containing the heated copper. In order to prevent oxygen from diffusing through this rubber connection, it was protected by a glass sleeve which was cemented in position.

Copper when heated to redness is rapidly oxidized by molecular oxygen. Consequently, if a gas containing a small amount of oxygen is passed over red-hot copper which had been completely reduced by hydrogen, the oxygen combines with the copper to form cupric oxide. In this instance a large excess of finely divided copper (about 1 pound of no. 60 mesh copper gauze) was used in order to insure a rapid chemical reaction; furthermore, the tank nitrogen was passed very slowly through the tube containing the hot copper. The

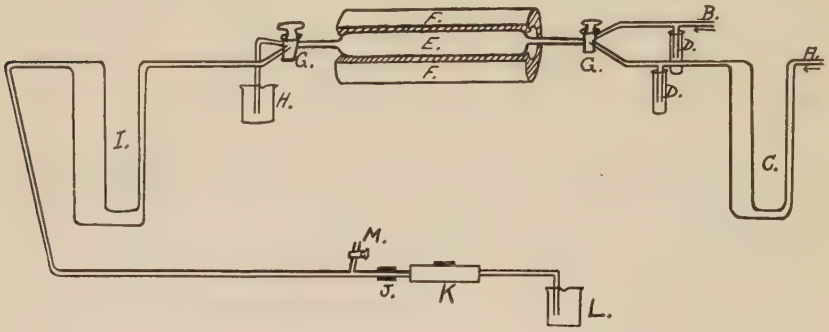

Fig. 10 Diagrammatic representation of the apparatus used to remove oxygen from tank nitrogen and hydrogen by means of hot copper. *A.*, intake from nitrogen tank; *B.*, intake from hydrogen tank; *C.*, wash tube containing sulphuric acid; *D.D.*, mercury safety wells; *E.*, tube containing copper gauze; *F.F.*, electric furnace, with side removed to show tube, *E.*; *G.G.*, three-way stopcocks; *H.*, water-sealed outlet; *I.*, wash tube containing redistilled water; *J.*, oxygen-tight connection between the glass and copper tubing; *K.*, observation chamber; *L.*, water-sealed outlet from observation chamber; *M.*, intake from oxygen tank.

tank nitrogen treated in this way will be referred to as oxygen-free nitrogen.

By this method it is comparatively easy to displace, from any system, the oxygen which is not in solution in a liquid, but to remove that which is in solution is quite a different matter. The oxygen that is used by amoebae must, of necessity, be dissolved in the liquid surrounding the animals. When a gas containing little or no oxygen passes over this, the oxygen passes out of the liquid by diffusion, until the oxygen tension in the liquid equals that in the gas passing over it. But how is one to know when this has occurred?

Several indicators which change color in the presence of oxygen were considered, but none of these proved to be satisfactory. The solution of this problem came when Prof. E. N. Harvey kindly supplied us with luminous bacteria. He says ('26):

> Absence of luminescence in Cypridina luciferin-luciferase or in luminous bacteria can . . . . be used as an indicator of lack of oxygen (the former is more delicate; bacteria begin to luminesce at an oxygen pressure = .0053 mm. Hg) . . . . mere removal of oxygen is sufficient for cessation of luminescence in both cases.

In order to test the efficiency of the method used in our experiments to remove oxygen from liquids, suspensions of luminous bacteria in sea-water were prepared. These suspensions were then put into the observation chamber, after which oxygen-free nitrogen was passed through the apparatus and the time recorded for luminescence to cease. All observations on these luminous bacteria were made in absolute darkness. The results obtained are as follows: 1) A concentrated suspension of the bacteria ceased to luminesce in four to eight minutes. 2) A thinner suspension ceased luminescence in nineteen to twenty-five minutes. 3) The thinnest suspension that could be seen in absolute darkness ceased in forty-five to fifty minutes. 4) Luminescence reappeared in one to three minutes after air or oxygen was again admitted to the chamber.

From these results it was concluded that, after an exposure of one hour to oxygen-free nitrogen, the oxygen was practically removed from the modified Ringer's solution in which amoebae were tested.

Three separate tests were made with oxygen-free nitrogen, the results of only one of which is presented in figure 11. In this test the rate of locomotion began to decrease almost immediately until it had reached 0.5 cm. per minute, at which time carbon-dioxide-free air was admitted to the observation chamber. A typical reaction to oxygen after oxygen-want occurred, which was followed by a very rapid return to normal. Upon recovery, the observation chamber was again

swept with oxygen-free nitrogen. This was again followed by a rapid decrease in the rate of locomotion. When oxygen was again admitted to the chamber, the animals recovered their former rate of locomotion, but not as rapidly as in the first part of this test. The reaction to oxygen after oxygen-want was not observed in the last part of this test.

By comparing figure 11, A, with figure 11, B, it can be seen that the gel/sol ratio decreased in lack of oxygen, and then

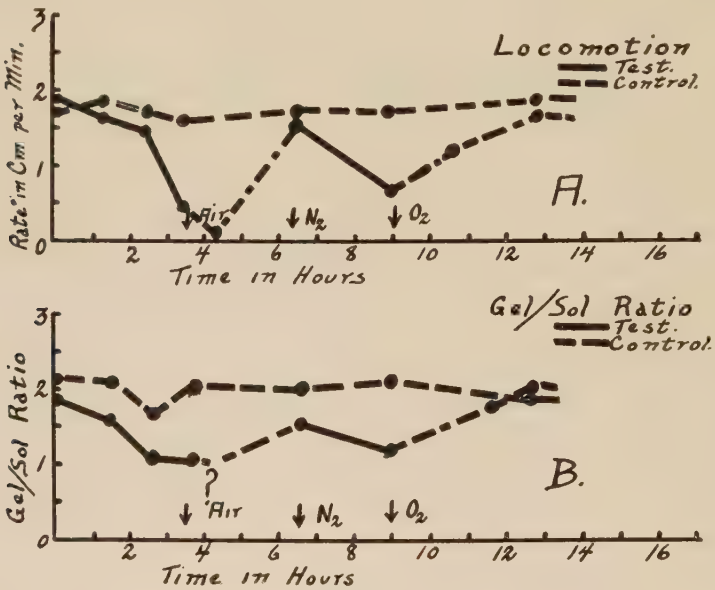

Fig. 11 Effect on Amoeba of displacing the air by oxygen-free nitrogen.

increased in air or oxygen, at almost the same time and at the same rate as did the rate of locomotion.

In the second test the rate of locomotion remained the same as that in air for almost two hours; then it decreased very rapidly, reaching zero at the end of three and one-half hours, but all movement of granules did not cease until the end of fourteen hours. At this time air was admitted to the observation chamber, with the result that the rate of locomotion during the following eight hours increased to slightly less than

that of the animals in air. However, only two of the experimental animals were alive at the end of this period. The reaction to oxygen after oxygen-want was not observed, but observations were not made at the correct time to observe this reaction.

The gel/sol ratio began to decrease almost immediately after the animals were exposed to lack of oxygen and continued to do so until it could no longer be ascertained. This ratio was essentially the same, in the two animals that recovered, as in the controls, when observed about eight hours after they had been restored to air.

If the oxygen was removed from the culture fluid in one hour, as indicated by the bacteria, then it is evident that locomotion can continue for several hours in the absence of oxygen, but at a diminished rate.

The results obtained in the third test are essentially the same as those obtained in the two preceding tests.

In all three tests typical pathological forms were observed. In the second test the animals passed through the entire series of forms as described in figure 3, with the exception that the process was much more rapid, requiring for the whole series only four to six hours; whereas it required about seventy hours in the test represented in figure 4. In the other two tests air was admitted before the effects were very severe, and therefore the animals did not pass through the complete series of pathological forms.

The rate of division was decidedly decreased by the reduction in oxygen pressure; only one animal divided in the observation chamber, while six divided in the control.

The following conclusions were drawn from these results; 1) In oxygen-free nitrogen both the rate of locomotion and the gel/sol ratio decrease very rapidly, and increase with equal if not greater rapidity if the animals are returned to air. 2) During the recovery period the characteristic response to oxygen after oxygen-want occurs. 3) The animals pass through the typical pathological condition much earlier than in air. 4) Lack of oxygen decreases the fission rate.

5) The effect of lack of oxygen is very similar to, if not identical with, that produced by greatly increasing the oxygen pressure. 6) Locomotion may continue for a considerable time in the absence of oxygen, but at a decreasing rate.

Oxygen-free hydrogen

The hydrogen used in this series of experiments was obtained from the Portsmouth Oxygen Corporation, Portsmouth, Virginia. It was prepared by electrolysis and its only impurity was a very small percentage of oxygen. This was removed by passing the tank hydrogen over red-hot copper in the apparatus described under "Oxygen-free Nitrogen." Thus the oxygen present combined with some of the hydrogen to produce water. After the hydrogen had passed over the hot copper, it was washed and conducted through the observation chamber. Hydrogen treated in this way will be referred to as oxygen-free hydrogen. It was tested for oxygen by means of luminous bacteria, as described above. The results obtained were practically the same as those obtained with oxygen-free nitrogen. It was therefore assumed that, after oxygen-free hydrogen had passed through the observation chamber for one hour, the oxygen was removed from the modified Ringer's solution in this chamber.

This series of experiments consisted of three separate tests, the results of one of which are presented in figure 12. In the first test (fig. 12, A) the rate of locomotion decreased extremely rapidly, reaching zero in one and one-half hours. The appearance of these animals was entirely different from that which obtained in almost all other experiments. This does not apply to every specimen exposed to hydrogen, but to some in every test. Soon after the air was replaced with hydrogen, there was a sudden reduction in both the amount and rate of streaming. Then the animals usually became well covered with hyaline blisters, some of which contained a few granules (fig. 13, A and B). These granules were always located at the center of the blisters. The appearance of the animals

would seem to indicate that the plasmagel contracted, forcing out the liquid ground-substance at different points, thus elevating the plasmalemma and forming the blisters (Mast, '26). After this, a considerable degree of adjustment to the hydrogen was exhibited in the majority of the animals. In this adjustment the granules slowly moved out into the hyaline blisters, ending in resumption of monopodal locomotion (fig. 13, D). Then they assumed successively forms similar to those observed in nitrogen (fig. 13, E, F, and G).

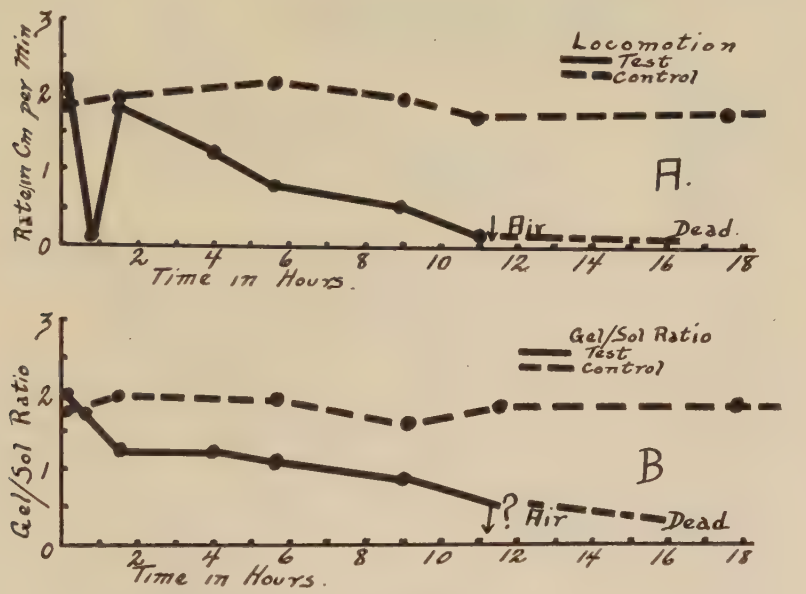

Fig. 12 Effect on Amoeba of displacing the air by oxygen-free hydrogen.

The first series of changes in form and structure described (entirely absent in nitrogen) were considered to be due to the action of hydrogen. The second series of forms (similar to those observed in nitrogen) were probably due to lack of oxygen.

There was a definite correlation between rate of locomotion and the gel/sol ratio similar to that observed in nitrogen (fig. 12).

Fig. 13 A series of camera-lucida sketches of amoebae in pure hydrogen, showing successive changes in structure and form. A and B. After exposure to hydrogen for one-half to one hour; no movement of granules, plasmalemma raised to form blisters, apparently due to action of hydrogen. C and D. Recovery after adjustment to hydrogen, after about two hours' exposure. E, F, and G. Changes in form probably due to lack of oxygen.

Similar results were obtained in the second and third tests. The rate of recovery after the hydrogen was replaced with air or oxygen was remarkably rapid in both of these tests. Changes in form and structure similar to those described above occurred in the third test. In the second test the reaction to oxygen after oxygen-want was observed, but observations were not made concerning the other changes in form and structure.

The following conclusions were drawn from the results of these three tests: 1) There is a marked difference between the action of nitrogen and hydrogen on Amoeba. 2) Hydrogen appears to have a direct and definite effect on amoebae aside from that produced by the elimination of oxygen, whereas nitrogen appears to act only through the elimination of oxygen.

EFFECT OF GREATLY DECREASED AND INCREASED OXYGEN TENSIONS ON THE HYDROGEN-ION CONCENTRATION IN THE CYTOPLASM IN AMOEBA

As a possible aid in understanding the pathological changes exhibited by the amoebae in almost every experiment, two of the above tests were conducted with animals lightly stained with neutral red. One of these experiments was with oxygen at 50 pounds' pressure, and the other was with oxygen-free nitrogen. No difference could be detected in the color of the control and the experimental animals at any time, except just before the death of the experimental animals. At this time the neutral red precipitated out and fine crystals were formed. This phenomenon occurs whenever an amoeba which has been stained with neutral red dies. It cannot, therefore, be considered as significant. It was concluded that, as far as can be observed, no change is produced in the hydrogen-ion concentration in the cytoplasm of amoebae subjected to very great changes in the amount of available oxygen.

DISCUSSION

It has been frequently suggested that the effect of oxygen at pressures above 1 atmosphere is due to the pressure exerted on the organism and not to the oxygen. This suggestion, however, is not valid, for Bert ('78), Rauber ('94), Samassa ('98), and others found no difference in the effect of air on animals under a wide range of pressure, and they obtained poisonous effects of oxygen by increasing the percentage of oxygen without changing the total pressure. Furthermore, it is very difficult to see how an increase in pressure could in any way affect organisms which contain no gas-filled cavities, as, for example, Amoeba.

The evidence presented in the preceding pages indicates that in Amoeba, high oxygen tensions and lack of oxygen produce the same effects. The question now presents itself as to the factors involved in the action of oxygen under the two conditions and how it is that high oxygen tension and lack of oxygen can produce the same effect.

Slater ('27), summarizing the oxidative processes occurring in muscle, says:

> The metabolic processes involved in the case of vertebrate muscle has been shown by Hill, Meyerhof and their co-workers to consist of two separate reactions, viz.; (1) an initial breakdown of glycogen to lactic acid, and (2) the transformation of a portion of the lactic acid back again into glycogen at the expense of the combustion of the remainder. The first reaction is anaerobic and is the source of energy in the organism, the second is aerobic, and serves simultaneously as a means of removing the lactic acid, and of conserving the energy available in the glycogen. The efficiency of the total process depends largely upon the ratio of the lactic acid returned to glycogen to the lactic acid burnt. During oxygen-lack the tissue obtains its energy in the normal manner, the lactic acid being allowed to accumulate. When oxygen again becomes available, the lactic acid is removed by synthesis and oxidation, the organism using an excess of oxygen, equal to that which it would have used during the period of anaerobiosis. In the case of vertebrate muscle, the ratio of lactic acid burnt to that resynthesised to glycogen is given by Furasawa and Hartee (1926) as 1 : 4.4, from which it can be calculated that a debt of 1 cc. of oxygen corresponds approximately to 6 mg. of lactic acid.

If it is assumed that amoebae derive energy for locomotion through processes which are essentially the same as the processes through which energy is derived in muscular contraction; then under normal conditions amoebae contain a given amount of glycogen in equilibrium with a small amount of lactic acid, and under anaerobic conditions this glycogen is changed into lactic acid which is not removed by synthesis and oxidation, and as the available amount of glycogen is used, the available amount of energy becomes less and less and consequently the rate of streaming must decrease. If this is true, then the time required for the rate of locomotion to increase to its former value is merely a period in which the lactic acid is being synthesized into glycogen.

An objection to this explanation is that, if amoebae are stained with neutral red and subjected to anaerobic conditions, no change in color is observed. This objection may be eliminated by assuming that the vacuoles which take the stain are filled with a fluid which is at least as well buffered as vertebrate muscle or the vital fluids from other organisms. If this is so, then the resulting changes in hydrogen-ion concentration would not be detectable with neutral red.

An explanation of the effects of increasing the oxygen tension is not as evident as in the case of lack of oxygen. Cleveland ('24) assumed that the poisonous effects of oxygen are due to rapid oxidation, and thus to actual burning of the protoplasm. In view of the results obtained in the experiments described above, it seems most unlikely that this can obtain.

If, however, the action is the same under both conditions, all that is necessary is to assume a decrease in the permeability of the outside layers of amoebae in high oxygen concentration. The same conditions would then obtain in the interior of the amoebae as are produced by complete absence of oxygen in the environment. That such a condition could occur in partially substantiated by the work of Faulkner, Binger, et al. ('27) on oxygen poisoning in mammals. They found that under high oxygen tensions the permeability of

the lungs to oxygen is decreased to such an extent that the animal actually suffers from oxygen deficiency. However, this detrimental effect of oxygen is produced only after long exposure and is counteracted by adding a small percentage of carbon dioxide to the oxygen. In our experiments the effect of the addition of carbon dioxide to oxygen was not ascertained.

This explanation would imply that the oxygen has a direct effect on specific parts of the organism and that in order to cause internal asphyxia without other injury to the interior of the cell this effect should occur in a rather brief period of time. The changes in permeability described above for lung tissue take considerable time, and Krogh has shown that (at least for brief periods of time) oxygen will pass rapidly through living tissues. The work of Krogh, Hopkins, Meyerhof et al. has shown that in all probability a definite oxidation-reduction potential is as essential for cell activity as is a definite hydrogen-ion concentration; and that this oxidation-reduction potential is maintained by the R-SH compounds of the cell, such as cystine, cysteine, and the different forms of glutathione. It would therefore seem more logical to assume that changing the oxygen tension over wide limits upsets this balance sufficiently to destroy the normal activity of the cell, possibly setting up entirely new chemical reactions within the cell.

SUMMARY

The rate of locomotion in Amoeba subjected to an atmosphere of pure nitrogen or hydrogen decreases rapidly, reaching zero in four to six hours; if the animals are then restored to atmospheric conditions, this rate again increases until, after a few hours, it has reached its former value.

Coincident with this decrease and increase in the rate of locomotion, the amount of plasmagel in relation to the amount of plasmasol decreases and increases, indicating a correlation between activity and the thickness of the plasmagel; i.e., high activity is correlated with thick plasmagel, and low activity with thin plasmagel.

As the rate of streaming and the amount of plasmagel decreases, the amoebae pass through a definite series of changes in form and structure which ends in death, if not stopped by restoring atmospheric conditions.

The rate at which division occurs is markedly decreased by subjecting the animals to insufficient oxygen.

Similar results are obtained if the oxygen is not completely removed, but is less than 0.005 atmosphere; however, the time required to produce these changes is much longer.

Identical changes in rate of locomotion, gel/sol ratio, form, structure, and rate of division occur if the oxygen pressure is increased to above 1 atmosphere. A pressure of 4.418 atmospheres of pure oxygen acts in the same way and in the same time as an atmosphere of pure nitrogen.

Changes in the amount of available oxygen from 0.005 to 100 per cent have no noticeable effect on Amoeba. Above or below these limits the animals are slowly killed, passing through identical pathological conditions.

Gaseous hydrogen appears to have a specific action on Amoeba besides the effect produced by reducing the amount of available oxygen.

Locomotion in Amoeba may continue for some time in the absence of oxygen, indicating that the energy processes here are similar to those found in the contraction of vertebrate muscle.

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VITA

Harold Raymond Hulpieu was born February 21, 1901, at Dodge City, Kansas. He completed his secondary education at Newton, Kansas, and entered Bethel College (Newton, Kansas) in 1919. After one year, he transferred to Southwestern College (Winfield, Kansas), where he majored in biology and chemistry. While at Southwestern College he was an assistant in general biology.

He spent the summer of 1922 at the Marine Biological Laboratory (Woods Hole, Massachusetts). At this time he accepted an assistantship in zoölogy at the University of Oklahoma (Norman, Oklahoma), under an arrangement whereby he received the degree of Bachelor of Arts from Southwestern College after one year. He received the degree of Master of Arts from the University of Oklahoma at the end of two years. The summer following this (1924), he held an instructorship in zoölogy at the University of Oklahoma.

He was assistant in general physiology at the Johns Hopkins University (1924–1925 and 1925–1926), and research assistant to Prof. S. O. Mast, at the Marine Biological Laboratory, during the summers of 1925 and 1926. During the year 1926–1927 and the summer of 1927 he was an instructor in biology at the North Carolina College for Women (Greensboro, North Carolina). He held an Adam T. Bruce fellowship in biology at the Johns Hopkins University during the year 1927–1928.